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## Selective Breeding Program for Genetic Improvement of *Macrobrachium rosenbergii* in Thailand

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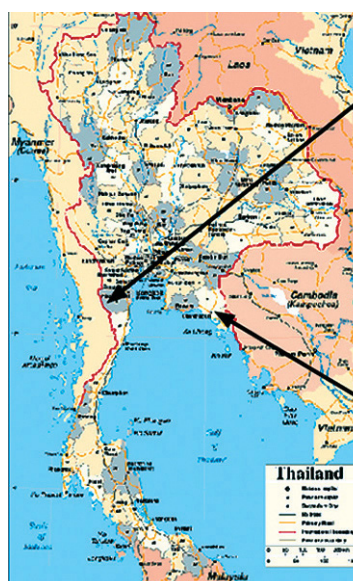
Dr. Panom Sodsuk and Dr. Supattra Uraiwan reporting the status of *Macrobrachium* selective breeding program in Thailand

Although the giant freshwater prawn (*Macrobrachium rosenbergii*) has been domesticated in Thailand for over decades, appropriate selective breeding program has yet to be achieved. Good quality seeds for the *Macrobrachium* aquaculture industry is therefore not regularly produced. One of the selective breeding programs on improving growth performance of the domesticated strain have been carried out at the Aquatic Animal Genetics Research and Development Institute (AAGRDI), Department of Fisheries of Thailand. AAGRDI has now developed improved and domesticated stock of *Macrobrachium rosenbergii* for two generations. Meanwhile, domesticated stocks from private hatcheries have also been acquired.

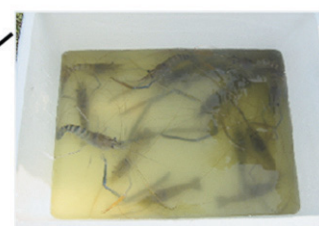
There is, therefore, the need to develop another improved stock of this species basically from these two domesticated stocks together with a wild stock in order to improve the genetic diversity of the base population for further selective breeding program. *Macrobrachium* wild stock has been domesticated under hatchery conditions at the AAGRDI for one generation. Generally, a good base population for genetic improvement program requires high genetic variation as well as an ideally suitable stock that can be well adapted for each of different local environments. Therefore, all proper crosses of these three stocks need to be cultured in different areas of the country and then evaluated on both performance and genetic variation before selective breeding program takes place.

The preliminary genetic studies that led to appropriate selective breeding criteria have been investigated in *Macrobrachium rosenbergii* by a group of researchers (Meewan, 1991, Uraiwan *et al.* 2002 and 2003; etc.). They estimated heritability on economic traits such as growth, body shape and morphotypic transformations, and they concluded that these traits have low to moderate estimated heritabilities. Therefore, the improvement of these traits can be carried out through selections. Uraiwan *et al.* (2003) reported that the within-family selection was possible to improve growth performance of *Macrobrachium rosenbergii* with the average response rate of 5-20 % per generation. Therefore, the present selective breeding program will use the within-family selection criteria.

Molecular technology at enzyme/protein level known as “allozyme marker” is widely accepted as a



Petchaburi farm



Chantaburi Province



powerful technique for studying genetic variation (Ward and Grewe, 1995), even the intra-specific population studies (Sodsuk, 1996; Sodsuk and Sodsuk, 1998a & 1998b; Sodsuk *et al.*, 2001). Due to the availability of the allozyme technique, it can be basically and initially applied in the genetic variation evaluation.

Among the DNA-based molecular technologies, microsatellite marker technique has a number of suitable qualities for genetic variation evaluation including (1) high polymorphism of many allelic gene loci, (2) co-dominant alleles inheritance with the homo/ heterozygous genotype of each individual identifiable, and (3) allele frequency and heterozygosity indicating amount of genetic variation obtainable (Queller *et al.*, 1993). Microsatellites display polymorphism by means of their variable numbers of tandem repeat motifs resulting in size variation, which can then be visualized by PCR with specific primers, followed by electrophoresis of the amplification product. The AAGRDI has been developing microsatellite markers for *Macrobrachium rosenbergii*, however the steps in optimizing PCR conditions for primers are needed in order that this technique could be used in addition to allozymes for evaluating genetic variation of further selective breeding program.

*The objectives of Thailand's selective breeding program are:*

1. To evaluate the economic traits performance and genetic variation of nine (9) crosses from three (3) stocks of *Macrobrachium rosenbergii*;
2. To improve economic traits of the best cross by suitable selection procedure;
3. To undertake PCR conditional optimization and primer test of microsatellite markers that have been developed for *Macrobrachium rosenbergii* by the AAGRDI; and
4. To apply polymorphism system of molecular markers (allozymes and/or microsatellites) in the genetic variation evaluation. (Allozyme markers will be basically and initially used, and microsatellites may be additionally applied later for further selective breeding program.)

*The activities of the program include:*

1. Allozyme analysis  
Genetic variabilities, observed heterozygosity and number of alleles per locus, of *Macrobrachium rosenbergii* from three populations are analyzed by allozyme electrophoresis.
2. Microsatellite markers
  - 2.1 PCR condition of microsatellite primers has been optimized considering the annealing temperature, amount of DNA template, MgCl<sub>2</sub>, primer and enzyme concentration, etc.
  - 2.2 The primers have been tested with a number of trials of screening *Macrobrachium rosenbergii* samples from different stocks using the optimized PCR condition.
3. Selective breeding program
  - 3.1 Reciprocal cross of three (3) stocks of *Macrobrachium rosenbergii* (1 wild, 2 domesticated stocks) to produce nine (9) crosses
  - 3.2 Performance evaluation to test between nine (9) crosses under three (3) environments (4 provinces: Pathomtanee, Utradit, Chomporn and Burirum). In each environment, all crosses will be reared together in three (3) ponds. The crosses are identified by the different colors, which have been injected into the prawn muscles.
  - 3.3 Genetic variation will be evaluated between nine (9) crosses based on allozyme markers.
  - 3.4 In each environment, the best performance cross will be chosen for the selective breeding program ( 4 environments may not be the same cross)

- 3.4 The within-family selection procedure will be used to improve the economic traits of the chosen cross.
- 3.5 Performance and genetic variation will be evaluated in each selected generation (later on, microsatellite markers may be ready to be used additionally to allozymes).
- 3.6 After three (3) generations of selection, the selected lines will be evaluated under the farm conditions.

#### ACTIVITIES: Year 2004-2005

Activities	2004												2005												
	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D		
• PCR conditional optimization and Allozyme analysis	←				→																				
• Primer test					←							→													
• Growing 3 strains and 9 crosses	←										→														
• Test on 4 environments (performance + genetic variation evaluation)									←				→												
• Apply suitable selection procedure on best crosses																←								→	
• Data analysis and report											←												→		

1. In June 2003, fifty pairs of *Macrobrachium rosenbergii* from each three stocks, namely NAGRI, Chantaburi and Petchaburi were collected to initiate the base population. Each stock has been spawned and reared separately. The offsprings of these three stocks have been reared in three 20 m<sup>2</sup> concrete ponds at the AAGRI.
2. Growth performances of *Macrobrachium rosenbergii* from three stocks have been observed during August 2003 to January 2004.
3. Allozyme electrophoresis has been carried out to estimate the genetic variabilities (heterozygosity and number of alleles per locus) of the three stocks.
4. Reciprocal cross of three (3) stocks has been initiated in July 2004 to establish nine (9) cross -lines

#### Progress of Activities

1. The allozyme analysis on the three (3) stocks is shown in Table 1 below:

**Table 1.** Observed heterozygosity and number of alleles per locus of *Macrobrachium rosenbergii* from three (3) stocks (NAGRI, Chantaburi and Petchaburi)

Populations	Sample size (No. of allozyme-loci screened)	Observed heterozygosity (mean ± sd)	No. of Alleles (mean ± sd)
NAGRI	25	0.043±0.018	1.360±0.11
Chantaburi	25	0.023±0.014	1.200±0.10
Petchaburi	25	0.036±0.016	1.440±0.13

There was no difference in the genetic diversity among the three (3) stocks ( $p>0.05$ ). In addition, the genetic variabilities of *Macrobrachium rosenbergii* from the present study are similar to those from



the previous study of natural stocks by Sodsuk and Sodsuk (1998b). [No of alleles 1.30 (1.29-1.33), heterozygosity 0.032 (0.027-0.036)]

2. Growth comparison between the three (3) stocks illustrated a growth difference between NAGRI and the other stocks as shown in Table 2 below:

**Table 2.** Mean length and weight of three stocks of *Macrobrachium rosenbergii* after six (6) months reared growout in concrete ponds

Stocks	Sex	Sample	Length (mean $\pm$ sd, cm)	Weight (mean $\pm$ sd, g)
NAGRI	Male	100	13.751 $\pm$ 0.704 <sup>b</sup>	31.047 $\pm$ 6.173 <sup>b</sup>
	Female	100	12.263 $\pm$ 0.845 <sup>b</sup>	20.355 $\pm$ 5.271 <sup>b</sup>
		200	13.007 $\pm$ 1.076 <sup>b</sup>	25.701 $\pm$ 7.842 <sup>b</sup>
Chantaburi	Male	100	13.310 $\pm$ 0.656 <sup>a</sup>	29.407 $\pm$ 12.538 <sup>a</sup>
	Female	100	11.724 $\pm$ 0.670 <sup>a</sup>	17.319 $\pm$ 6.238 <sup>a</sup>
		200	12.517 $\pm$ 1.034 <sup>a</sup>	23.363 $\pm$ 11.588 <sup>a</sup>
Petchaburi	Male	100	13.405 $\pm$ 0.882 <sup>a</sup>	28.490 $\pm$ 5.769 <sup>a</sup>
	Female	100	11.520 $\pm$ 1.027 <sup>a</sup>	15.159 $\pm$ 3.183 <sup>a</sup>
		200	12.463 $\pm$ 1.343 <sup>a</sup>	21.824 $\pm$ 8.139 <sup>a</sup>

\* The different letter illustrates statistical differences at p-value p<0.01

It is concluded that *Macrobrachium rosenbergii* from the NAGRI stock are larger by 4% in length and 9-15% in weight than those of the Chantaburi and Petchaburi stocks.

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